Validation of two internationally standardized detection methods (*Peronospora* and *Stagonosporopsis valerianellae*) on corn salad seeds for ISHI-Veg

Geoffrey ORGEUR[1] Juliette DESLILES[2] and Valérie GRIMAULT[1]
1GEVES, SNES, Laboratoire de Pathologie, 25 rue Georges Morel, 49071 Beaucouzé, France
2 HM Clause, Rue Louis Saillant, ZI la Motte, BP83, 26802 Portes-Les-Valence cedex, France

Contact: geoffrey.orgeur@geves.fr

Introduction, context and objectives

Several detection methods for *Peronospora* (Pv) and *Stagonosporopsis valerianellae* (Sv) exist in the seed industry. Some are based on a grow-out (germination on substrate and observation of symptoms for Pv or observation of suspect colonies on media or blotters Sv). Following a comparison, standardized ISHI-Veg methods for the detection of Pv and Sv in corn salad seed were developed.

Mat & Methods

Various technical comparison of modalities for each protocols were compared (watering, sowing, environmental conditions, characterization of typical infected plantlet for Pv and selectivity of the media, characterization of the typical suspect colonies, pathogenicity test and PCR confirmation for Sv) in order to optimize the method. Analysis of the performance criteria (diagnostic sensitivity and specificity, selectivity reproducibility and repeatability) were carried out in order to organize an international validation ring test. Each participant received 3 level of infected seed lots (healthy, medium and high) with at least 3 samples per lot.

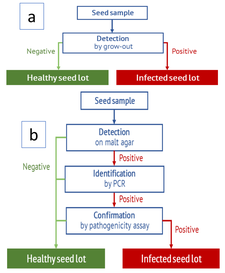

Fig. 1 Workflow of detection method on Pv (a) and Sv (b)

Methodological comparison

***Stagonosporopsis valerianellae* on media**

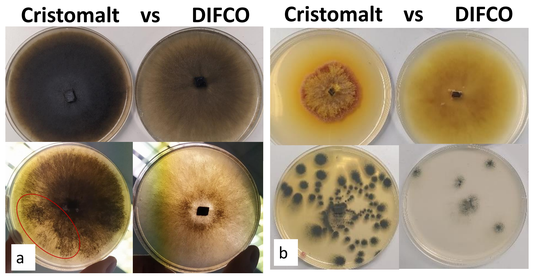

Fig. 2 Comparison of different media on Sv (a) and saprophytes (b)

Difference on morphology on Cristomalt :
- Mycelium thick
- Intense color
- Pycnidia sporulation at D+14

***Peronospora valerianellae* by grow out**

Fig. 3 Comparison of different sowing, hygrometry and temperature on Pv

- Sample: 400 seeds in 1 container
- Volume of water defined→100 mL / Kg of soil
- Density of sowing defined→ 1 seeds / 1.5 to 2 cm2
- Temperature at 12° C better than at 14° C

Participants and results

Tab. 1 listing of participants

Participants	Sv	Pv
Lab 1	X	X
Lab 2	X	X
Lab 3	X	X
Lab 4	X	X
Lab 5	X	

Different seed companies and institutes' laboratories were volunteers to participate to these international validations comparative test ongoing in 2021-2022. *Peronospora* is still ongoing and results are not analysed.

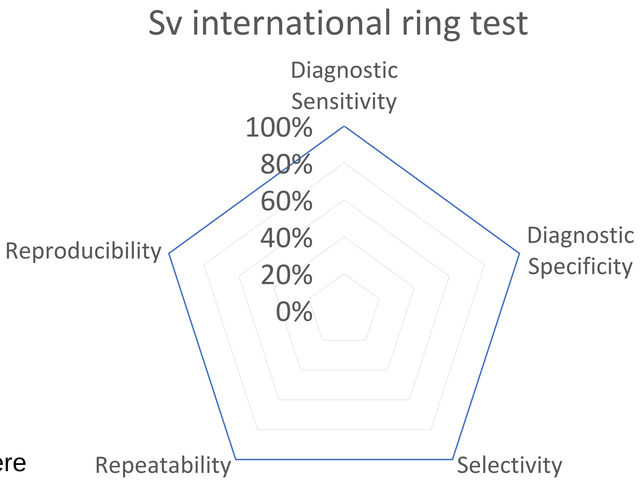

Fig. 4 Performance criteria on Sv

Results obtained on Sv are characterized by diagnostic sensitivity and specificity at 100% and confirmed that all the results obtained corresponded to expected results : in total 15 samples (5 participants x 3 rep) obtained negative results on the healthy seed lot and 30 samples (5 participants x 3 rep x 2 seed lots) obtained positive results on the 2 infected seed lots. In parallel, the assay on 8 repetitions showed also 100% of repeatabilty, and selectivity (tested on different varieties from different origins). At last, the results obtained from all participants allowed to confirme a reproducibility at 100 %.

Conclusions and perspectives

The qualitative analysis on Sv ring test showed a very high level (100%) of diagnostic sensitivity and specificity, as well as repeatability and reproducibility. In parallel, two confirmation methods (pathogenicity test and PCR) are developed, validated and available to confirm *Stagonosporopsis valerianellae* identification.

The international ring test to detect Pv by grow out protocol is still under progress, but the first results received looked in accordance with the expected ones. The same analysis will be done in a few weeks and published to ISHI-Veg in order to validate both methods.